Species Composition and Toxicity of By-catch Crabs in the EEZ Sarawak

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Bachelor of Science with Honours
(Aquatic Resource Science and Management)
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Siti Nur Ashieqa Binti Hasli

This dissertation is submitted in partial fulfilment of the requirement for the degree of Bachelor with Honours in Aquatic Resource Science and Management

Faculty of Resource Science and Technology
University Malaysia Sarawak
2016
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I, Siti Nur Ashieqa Binti Hasli, declare that the final year project report entitled:

**Species Composition and Toxicity of By-catch Crabs in the EEZ Sarawak**

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- none of this work has been published before submission

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Date: 27/6/2016
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<tr>
<td>PSP</td>
<td>Paralytic Shellfish Poisons</td>
</tr>
<tr>
<td>PST</td>
<td>Paralytic Shellfish Toxins</td>
</tr>
<tr>
<td>TTX</td>
<td>Tetrodotoxin</td>
</tr>
<tr>
<td>STX</td>
<td>Saxitoxin</td>
</tr>
<tr>
<td>EEZ</td>
<td>Exclusive Economic Zone</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>Alumina (Aluminium oxide)</td>
</tr>
<tr>
<td>SiO₂</td>
<td>Silica gel (Silica dioxide)</td>
</tr>
<tr>
<td>AcOH</td>
<td>Acetic Acid</td>
</tr>
<tr>
<td>KOH</td>
<td>Potassium Hydroxide</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium Hydroxide</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>Rₖ</td>
<td>Retention Factor</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>CPUE</td>
<td>Catch per unit effort</td>
</tr>
<tr>
<td>MU</td>
<td>Mouse Unit</td>
</tr>
<tr>
<td>CW</td>
<td>Carapace width</td>
</tr>
<tr>
<td>CL</td>
<td>Carapace length</td>
</tr>
<tr>
<td>ND</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>
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Siti Nur Ashieqa Binti Hasli

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ABSTRACT

One hundred and sixty four samples of crabs were collected by-catch from 35 stations in EEZ Sarawak, during cruises of M. V. SEAFDEC 2, 18 August – 17 September 2015. The crab samples were collected using otter trawl net with the depth of capture range from 22 m to 140 m depth. Seven different Families, composed of 23 different species were identified based on morphology characteristics. The composition of crabs at each station were recorded. The species frequently found predominant in each Leg were Charybdis miles, Charybdis acutidens and Calappa spp. While for toxicity studies, 10 species were extracted and analyse to assess their toxin proprieties. Among the ten species analysed for Saxitoxin (STX) using Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) none of the samples showed positive results indicating these species does not contained STX as the major toxins. However, for tetrodotoxin (TTX) analysis using LC-MS/MS analysis, two of the species are classified as weakly toxic and one of the species being classified as moderately toxic. The data collected in this study will be the basis of crab composition that using trawl and provide useful toxicity data of crabs in Sarawak waters.

Keyword: Crab, By-catch, EEZ Sarawak, Saxitoxins, Tetrodotoxins

ABSTRAK

Seratus enam puluh empat sampel ketam telah diperolehi melalui tangkapan bukan sasaran daripada 35 stesen di perairan EEZ Sarawak, semasa pelayaran M. V. SEAFDEC 2, 18 Ogos- 17 September 2015. Sampel-sampel ketam telah ditangkap menggunakan pukal tunda di mana kedalaman menangkap bermula dari 22 m sehingga 140 m kedalaman. Tujuh keluarga ketam yang terdiri daripada 23 spesies yang berbeza telah dikenalpasti berdasarkan karakter morfologi. Komposisi ketam bagi setia stesen direkod. Spesis utama yang kerap ditemui ialah Charybdis miles, Charybdis acutidens, dan Calappa spp. Manakala bagi kajian ketoksisan, 10 spesis telah diekstrak dan dianalisis untuk menilai ciri-ciri toksin mereka. Antara 10 spesies yang telah dianalisis bagi saksitoksin (STX) menggunakan kaedah Kromatografi Lapisan Nipis (TLC) dan Kromatografi Cecair Tinggi (HPLC), tiada sampel yang menunjukkan keputusan positif menunjukkan spesies-spesies ini tidak megalang STX sebagai toksin utama. Bagaimanapun, bagi analisis tetrodotoxins (TTX) menggunakan analisis LC-MS/MS, dua spesies telah dikelasifikasi sebagai bertoksik lemah dan satu spesis diklasifikasi sebagai bertoksik sederhana. Data yang diperoleh melalui kajian ini dapat menjadi asas komposisi ketam yang ditangkap menggunakan pukal dan menyediakan data toksisiti yang berguna bagi ketam-ketam di perairan Sarawak.

Kata kunci: Ketam, Tangkapan bukan sasaran, EEZ Sarawak, Saksitoksin, Tetrodotoxins.
1.0 Introduction

Crabs are crustacean which can be found in most marine habitats in Malaysia, such as in, coral reefs, sandy beach, rocky beach, lightless abyss as well as on dry land and many freshwater biota. Crabs are one of the most important coastal aquatic species worldwide because of its high consumer demand that leads to fetch a high price both in local and export markets (Chitravadivelu, 1994).

Savad and Rahavan (2001) stated that, crabs rank third after shrimps and lobsters for their esteemed food delicacy and also the value of fishery they support. In addition, crabs provide good source of protein to man as well as marine life. Crab meat is relished as an exclusive and rich in vitamins. The shell and flesh of crabs are high in its protein content as compared to mollusc (Ackman, 1990). It was discovered that, 20 different classes of amino acid were found in shells and tissues of some crab and the crab flesh alone can supply all the amino acids required for growth (FAO, WHO & UNO, 1985). Hence, it is believed to carry therapeutic qualities for colds, asthma and wheezing (Tharmine et al., 2014).

Different crab species inhabit difference area depending on the food availability and a place for them to shelter. Distribution of crabs are mostly related to salinity (Ysebaert et al., 2003), sediment characteristic (Anderson et al., 2004), temperature, ions and water availability (Wolcott, 1988), pH and topography (Sherman, 2003). Cob et al. (2012) indicated that, most crabs hide in rock, under vegetation or by burrowing into soft sand or mud. They also inhabit sheltered or exposed reef and seagrass or seaweed bed.

While most crab are edible, some of them have toxic properties which make them are dangerous to consume. Serious threat and hazards could lead to people being defenceless through ingestion of inadequately cooked crabmeat by the presence of toxicity (Okonko et al., 2009). Hwang and Tsai (1999) stated that, the first case of human intoxication due to seafood consumption in Malaysia was recorded in May 1978 at Sabah, where there were
seven numbers of deaths while another 196 persons suffered from illness due to paralytic shellfish poisoning (PSP).

Moreover, although several studies have been done regarding crabs in Malaysia, limited studies of crab composition have been conducted especially in Sarawak waters. There are less information on crabs composition being provided on types of crab species inhabit at certain area such as in Sarawak. Furthermore, there are also food poisoning cases due to consumption of crustacean which some of them lead to human and animals death (Hashimoto et al., 1967). Therefore, the aims of this study were to study the current crab’s composition in Sarawak waters and to screen toxicity of selected crab species.

In this study, the samples were bycatch samples and were collected by using otter trawl net using M. V. SEAFDEC 2 cruise at Exclusive Economic Zone (EEZ), along Sarawak waters from Miri until Kuching. By-catch means these species were caught unintentionally while catching certain target species and target sizes of fish. The types of crab species present at each station were listed for documenting the species composition while for toxicity studies, 10 species were selected to analyse to assess their potential danger using Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), and LC-MS/MS Analysis methods. These methods were conducted to detect the present of toxins which were tetrodotoxin (TTX) and saxitoxin (STX) available in the crabs.

Therefore, the objectives of this study were 1) to study and identify the composition of by-catch crabs in EEZ Sarawak Waters, and 2) To identify and determine the toxicity level by using the High Performance Liquid Chromatography (HPLC) and LC-MS/MS Analysis methods on selected crab species.
2.0 Literature Review

2.1 Crabs Taxonomy

Phylum : Arthropoda
Class : Crustacea
Order : Decapoda

Crabs belong to Phylum Arthropoda, Class Crustacea and Order Decapoda (Gopalakrishnakone, 1990). Some of the crab family found in this study are Family Calappidae (box crabs or shame-faced crabs), Family Goneplacidae, Family Portunidae (swimming crabs), Family Dromiidae, Family Homolidae (Carrier crabs) and Family Parthenopidae.

2.1.1 Classification and Morphology of Crab

Both male and female crab morphologies are different from each other. In order to distinguish their sex, there are specific classification were used such as by observe the shape on their abdomen (James, 2009). Male crabs usually have 'T' shaped or triangular abdomen while female crab’s abdomens are broad or ‘U’ shaped and usually semicircular and often covering most part of the ventral surface (James, 2009)(Figure 1). Crabs body consist of head, thorax and abdomen where the head and thorax fused to be cephalothorax and covered by chitinous exoskeleton called carapace (Wisespongpond, 2001). Different crabs may have different morphology (Herter et al., 2011). Some species might have almost similar morphology which make it difficult to distinguish it from one another. Crabs can be classified into two main groups which are Brachyuran crabs (true crabs) which have 4 pairs of well-developed walking legs and Anomuran crabs which have only 3 pairs of visible
walking legs while the fourth pair is very small and hardly noticeable (Wisespong pand, 2001). From this study, the crabs obtained were Brachyuran crabs. Table 1 shows the classification of crabs based on their morphology structure.

![Figure 1: The differences between male and female crab](adapted from FAO, 2002)

<table>
<thead>
<tr>
<th>Family</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calappidae</td>
<td>Sides of carapace may be expanded to form a clypeiform process. Right chela (rarely left) with specialized cutting tooth, the fingers of other chela long, forceps-like; propodus and dactylus of pereopods 2-5 never paddle-like.</td>
</tr>
<tr>
<td>Goneplacidae</td>
<td>Male abdomen distinctly triangular, with lateral margins of somites 3-6 distinctly converging towards telson. Abdominal somite 3 about 2 times telson width.</td>
</tr>
<tr>
<td>Portunidae</td>
<td>All have a sub-oval carapace with well-developed teeth on the anterolateral margins. Swimming crabs have the dactylus of which is paddle-shaped in most species.</td>
</tr>
<tr>
<td>Dromiidae</td>
<td>Carapace circular to hexagonal. A small platelet-like structure intercalated between edges of abdominal somite 6 and telson. Crab carries sponges or related objects.</td>
</tr>
<tr>
<td>Homolidae</td>
<td>Carapace longitudinally rectangular, dorsal surface glabrous or with scattered stiff setae. Only pereopod 5 with dactylus and propodus subchelate to chelate.</td>
</tr>
<tr>
<td>Parthenopidae</td>
<td>Press button on sterno-abdominal cavity consisting of a low peg-like tubercle on anterior edge of sternite 5. Male abdomen relatively broad.</td>
</tr>
</tbody>
</table>
Latreilliiidae Basal segment of eyestalk much longer than terminal article, from dorsal view, eyestalk appears to be 2 segmented.

2.2 Crabs Composition in Malaysia

The late Raoul (1968), estimated that perhaps some 1 000 species of brachyuran crabs occur in the Indo-Malayan area. However, due to the rapid pace of crab discoveries, these numbers have significantly increased over the last 40 years. Very limited studies had been done regarding the crabs composition in Malaysia. Most study emphasised on specific types or species such as studies on mud crabs, coral crabs, fiddler crabs and others. Recently, fieldwork done by researcher from Netherlands Naturalis Biodiversity Centre in Indonesia and Malaysia lead to the discovery of a new coral-dwelling gall crab. The new gall crab, named *Lithoscutus semperi*, was discovered inhabiting free-living corals of the species *Trachyphyllia geoffroyi* on sandy bottoms near coral reefs (Van der Meij, 2015).

A report by Agrodev Canada Inc. for a fisheries report for Sarawak on 1993 stated that, there are two main Brachyuran crabs which were commercially harvested. One of them is *Portunus pelagicus*. In 1997, the Annual Fisheries Statistics for Sarawak mentioned that these two Brachyuran species were commercially crucial.

2.3 Crabs Toxicity Cases

The first case of human intoxication due to seafood consumption in Malaysia was recorded in May 1978 at Sabah, where there were seven numbers of deaths while another 196 persons suffered from illness due to paralytic shellfish poisoning (PSP) (Hwang and Tsai, 1999).

A study by Yasumura *et al.* (1986), discovered 15 marine crab species were screened for lethality by mouse assays to assess their potential danger. However the 15 marine crab species were not mentioned in this study. Because of its frequent implication in human
intoxication, particular attention was paid to the determination of the toxic principle in *Zosimus aeneus*. Inoue *et al.* (1968) subsequently found the toxin of *Atergatis floridus* to be similar to that of *Z. aeneus* in chromatographic behaviour and pharmacological action in mice, resulting evidently indicates that both toxins were the same chemical compound. Crab toxin showed a similarity in pharmacological actions to tetrodotoxin (TTX) and saxitoxin (STX), suggesting a modification of experimental techniques to follow more closely those used for these toxins.

Hashimoto *et al.* (1967) reported two cases of crab poisoning in which both human and animal victims were died. One of the two case happens in 1928, where two person are dead while the other occasional outbreaks of crab poisoning resulting in 12 fatalities on Negros Island, Philippines. The lethal potencies of the following five species have been reported which are *Z. aeneus*, *A. floridus*, *Lophozozymus pietor*, *Demania toxica* and *Demania alcalai* (Carumbana *et al.*, 1976).

2.4 Toxin properties

Kao (1966) mentioned that saxitoxin (STX) and its analogs (STXs) are potent neurotoxins that block voltage-gated sodium channels on the cells. Paralytic Shellfish toxins are initially produced by the eukaryotic dinoflagellates in the marine environments, which belong to the genera *Alexandrium*, *Gymnodinium* and *Pyrodinium* (Lefebvre, 2008; Usup, 1994). The toxins are passed through the marine food web via vector organisms, which accumulate the toxins by feeding on PST producing dinoflagellates without apparent harm to themselves (Gainey *et al.*, 1988; Shumway, 1995). These include filter feeding invertebrates such as shellfish, crustaceans, molluscs and also other, non-traditional vectors such as gastropods and planktivorous fish.
While, TTX is a very potent neurotoxin that is found in a variety of marine and also in some terrestrial. The mechanism of TTX toxicity has been studied in animal models (Saoudi et al., 2007; Hasan et al., 2008; Zimmer, 2010). It is a sodium channel blocker. The toxin will binds to the sodium channels of the excitable tissues of the victim (muscles and nerves), then the inhibition of sodium ions through the channels effectively immobilises these tissues (Denac et al., 2000). On an equal quantitative scale, TTX is claimed to be 10000 times more lethal than cyanide and it is one of the deadliest poisons on earth (Keyvan, 2004). The lethal potency of the crabs was expressed as mouse units per gram of crab (MU/g), where 1 MU was define as the amount of toxic material required to kill a mouse of 20 g body weight in 15 minute (Daisuke et al., 1985).

2.5 High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) was developed in the late 1960s and early 1970s. Kupiec (2004) reported that, HPLC is a form of liquid chromatography used to separate and quantify compounds that have been liquefied in solution. By using HPLC method, volume of a specific compound in a solution could be determined. For example, in toxicity study, tetrodotoxin (TTX) and saxitoxin (STX) compound can be analysed. Furthermore, to avoid excessive killing of mice and achieve high sensitivity and specificity in TTX monitoring, Yasumoto et al. (1984), and Yotsu et al. (1989) constructed an identical fluorometric analyser, by combining HPLC and a post column reaction with a hot NaOH solution, to detect TTX and its derivatives.
3.0 Materials and Methods

3.1 Sampling Sites

The samples were collected at Exclusive Economic Zone (EEZ) Sarawak which comprise from Miri area to Kuching area. Figure 2 shows the location of the sampling station while Table 2 showed the location of the trawling and the number of station for each Leg. In addition, Appendix A showed the details location of stations with its coordinates.

Figure 2: The locations of the sampling station. Marks represent sampling locations.
Table 2: The location of the trawling and the number of station for each Leg

<table>
<thead>
<tr>
<th>Leg</th>
<th>Number of Station</th>
<th>Locations</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>8</td>
<td>Miri – Bintulu</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>Bintulu- Bintulu</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>Bintulu – Kuching</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>Kuching – Kuching</td>
</tr>
</tbody>
</table>

(a) legs 3 [Station 145, 152, 143, 138, 99, 49, 50, 48] (b) legs 4 [Station 59, 58, 93, 94, 92, 89, 86, 82, 83, 88, 137, 135, 134, 80, 85, 81, 56, 57, 45 and 46], (c) Legs 5 [Station 132, 131, 130 and 127], and (d) legs 6 [Station 22, 63 and 126]. Marks represent sampling locations.
3.2 Field Work

The collaborative studies were carried out on board M. V. SEAFDEC 2 from 28 July 2015 to 4 August 2015. The by-catch crab samples were collected from 35 stations in EEZ Sarawak waters, during 18 August 2015 to 17 September 2015. Overall, 164 crabs samples were collected by otter trawl net by M. V. SEAFDEC 2 cruise. The samples were obtained from 4 different Legs which were Leg 3, Leg 4, Leg 5 and Leg 6 which comprise 35 stations with the depth range until 200 meter depth. In this study, a standard trawler was used by the Fisheries Research Institute Bintawa Sarawak to collect crabs samples and few other samples. The net mesh size used range from 1 inches, 2 inches and 4 inches. Then, the crabs collected were placed in the labelled plastic bags and preserved in freezer. The collected samples were brought back to the laboratory for species identification and basic toxicity studies.
3.3 Laboratory work

3.3.1 Morphometric Measurement

The external carapace of the crabs were measured for the carapace length (CL), which was the distance from the median frontal teeth to the the posterior border of the carapace, and carapace width (CW) which was the distance taken between the widest point of the carapace or between the two tips of anterolateral (Lai et al., 2010; Ikhwanuddin et al., 2012) (Figure 3). The CL and CW were measured to the nearest 0.1 mm. While the body weight (BW) of all crabs were measured using electric analytical balance to the nearest 0.01 g.

![Figure 4: The structure of carapace shape (adapted from FAO, 2002).](image)

3.3.2 Crabs Identification and Classification

The crabs were sort out according to their stations and the samples were further identified using key to species identification by Ingle (1997), Dai and Yang (1991), Castro (2007) and Ng et al., (2009). The morphology characteristic were observed to identify the crabs. Some of the morphology characteristics been observed were the shape of carapace, shape of chelipeds, number of spine, (either it is with two obvious spines or without two obvious spines), the position of frontal lobe (high or low) and the number of pereiopodes. Besides,
there are also many types of carapace shape that is usually used as a descriptive character in many guide and keys including hexagonal, transversely ovate, squarish and trapezoidal (Ingle, 1997)

3.3.3 Data Analysis

Crab for each station from four different Leg were counted. For documenting the crab composition, the crabs were listed according to their stations, species, number of crabs sample for each species, sex, carapace length, carapace width and their weight. Since the crabs were by-catch samples, the diversity index and species richness index were not used in this study since it was non-target species and it did not represent the whole crab community at the particular areas.

3.3.4 Toxin Extraction

The extraction method in this study was done by followed the A.O.A.C. standard method. Firstly, the specimens were partially thawed and divided into the appendages, cephalothorax and internal organs parts. Each body part were minced and grinded by using scissor, blade, and mortar. Two gram of tissue were extracted with an equal volume of 6 ml of 0.03 M acetic acid (AcOH). Then, the mixture was heated in a boiling water bath for 10 minutes and after that being cool down with ice cubes for 10 minutes before it was centrifuged at 8,000 rpm for 30 minutes. After that, the supernatant or extracted toxin was pipetted using syringe along with syringe filter and placed in centrifuge tube and further stored in the refrigerator with -20°C before it was used to perform Thin Layer Chromatography (TLC).
3.3.5 Thin Layer Chromatography

The extracted toxins were spotted onto silica gel-60 F254 pre-coated plate (Merck). For each plate, the TTX and STX standard were spotted at the first point as a marker. The plate was spotted with three point which represent three parts of the crabs that are appendages, cephalothorax and internal organ parts. The plate then was developed into eluent contain butanol-acetic acid-water (2:1:1). The eluent which is also called as solvent rises through capillary action and ascending chromatographic separation was obtained. The plate then dried and viewed under UV light (UVGL-55 Handheld UV Lamp, 365 nm). Then, the visualized spots were sketch by using a pencil. Retention factors $R_f$ of the spot was calculated by dividing the distance travelled by the solvent front (Asakawa et al., 2002).

3.3.6 High Performance Liquid Chromatography (HPLC) Analysis

Reversed phase HPLC was performed on Water 600 Cooler system by using Symmetry C18 5 μm (4.6 x 150mm) column for STX and TTX analysis. The HPLC condition were shown briefly in Table 3. For this study, two types of buffers were used which were 60 mM ammonia phosphate (pH 5) and 4 M NaOH (sodium hydroxide). To prepare 60 mM ammonium phosphate, 300 ml Milli-Q water was mixed with 205 μl phosphoric acid. Then, 1.0112 g of heptanesulfonic acid (HAS) was added to the mixture followed by Milli-Q water until the volume reached 500 ml. After that, 10 ml of acetonitrile was added to the mixture. Lastly, the mixture were filtered with 0.45 μm Milipore filter paper while 4 M of NaOH was completed by dissolved 74.32 g of sodium in solid form with 500 ml Milli-Q water (A.O.A.C., 1995).

Table 3: Operating conditions of HPLC for the analysis of STX and TTX

<table>
<thead>
<tr>
<th>HPLC system</th>
<th>: Water 600 Cooler</th>
</tr>
</thead>
<tbody>
<tr>
<td>For TTX analysis:</td>
<td>: 60 mM ammonia phosphate (pH 5)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>14</td>
</tr>
</tbody>
</table>

Reagent: 4 M NaOH
Flow rate: 0.8 mL/min
Reaction temperature: 110°C
Detection: Excitation 381 nm, emission 505 nm
For STX analysis:
Mobile phase: 0.1 M Na 1-heptanesulfate (pH 7.0)
: 0.5 M H₃PO₄
: Distilled water
: CH₃CN
Reagent: 7 mM periodate
Acidifier: 0.5 M acetic acid
Flow rate: 0.8 mL/min
Reaction temperature: 65°C
Detection: Excitation 330 nm, emission 390 nm

3.3.7 Liquid Chromatography Mass Spectrometry (LC-MS/MS Analysis)

LC-MS/MS used the standard method by Diener et al. (2007) by using TSQ Quantum Discovery MAX (Model Thermo Electron, USA) with calibration 1, 3, 5 polityrosine in both positive and negative modes. The operating conditions were voltage of 3800 V, sheath gas flow of 10 units, auxiliary gas flow of 3 units, collision energy of 18, collision gas pressure of 1.5 mTorr and capillary temperature of 300°C.

While, the software that will be used was Xcalibur 2.1.0 to detect peak, calibration and data acquisition of graph plot 5μm (150 mm x 2.1 mm inner diameter) ZIC-HILIC column (SeQuant Haltern, Germany), guard column of 5 μm (20 x 2.1 mm)(SeQuant Haltern, Germany) and eluent flow rate of 250 μL min⁻¹ were used to separate TTX. The mobile phase A used were 10 mM ammonium formate and 10 mM formic acid while 5 mM ammonium formate and 2 mM formic acid were used as mobile phase B in acetonitrile water (80/20, v/v).

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4.0 RESULTS

4.1 Crabs Identification

4.1.1 Species Composition

The crabs were collected from 35 station which is from four Leg that were Leg 3, 4, 5, and 6. A total of 22 species was analysed from 164 crab samples. The total number of crabs found for each species and percentage occurrence of by-catch crab’s family were summarize in Table 4. While species occurrence at particular station and legs were presented Appendix B, C, D and E.